Effective synthesis and pharmacological evaluation of 1,2,4-triazolo-isoxazole and 1,2,4-triazolo -pyrazole scaffolds

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Abstract
Five-membered heteroaromatic compounds like pyrazoles and isoxazoles are significant for the pharmaceutical sector and material research, becoming more significant in drug research, cosmetics as well as in the creation of agricultural products. 1,2,4-triazolo fused isoxazole derivatives (3a-f) were synthesized from reaction between intermediate chal cone-1,2,4-triazolo-1,5-benzodiazepine derivatives 2a-f with hydroxyl amine hydrochloride. 1,2,4-triazolo fused pyrazole derivatives (3a-f and 4a-f) were prepared from reaction between chalcone derivatives 2a-f with hydrazine hydrate and its phenyl derivatives respectively in high yields.

Synthesized isoxazoles and pyrimidine derivatives have broad spectrum of medicinal importance. These pharmacophoric scaffolds represent a class of anti-microbial screening for Candida albicans, Staphylococcus aureus, Enterobacter cloacae, Fusarium oxysporum and Escherichia coli.

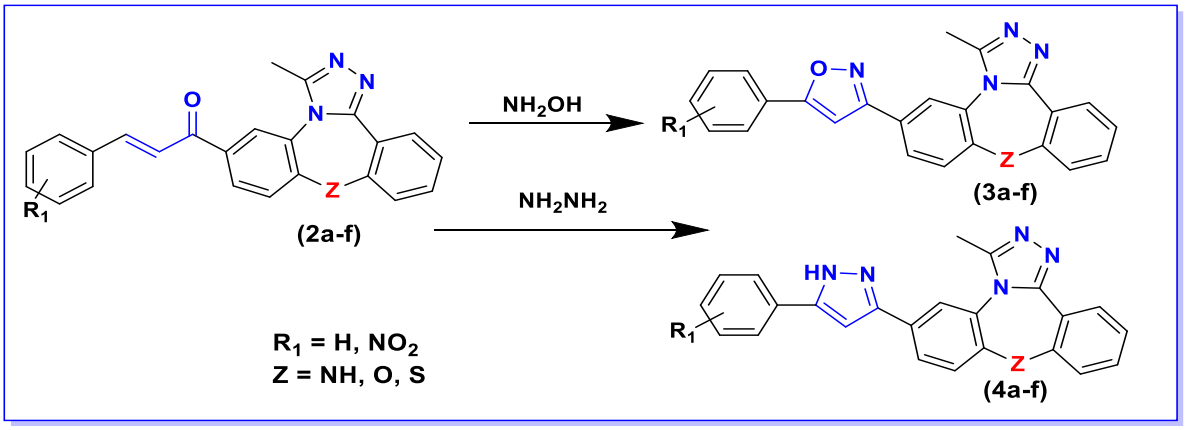
Keywords: Cyclocondensation, Pyrazoles, Isoxazoles, Chalcones, 1,2,4-triazolo derivatives, Bezodiazepines, Benzothiazepines and Benzoxazepines.

Introduction

The chemical behavior of 1,2,4-triazolo-pyrazoles and 1,2,4-triazolo-isoxazoles is distinct from that of other related triazoles as well as heterocyclic compounds in general. A review of the literature reveals that adding bioactive pharmacophores of 1,2,4-triazolo-pyrazoles and 1,2,4-triazolo-isoxazoles to the current therapeutic molecules significantly enhance the drug's overall biological characteristics[2]. This observation raises the possibility that novel analogues with improved biological profiles could be produced by adding 1,2,4-triazolo fused isoxazole and pyrazole pharmacophores to the 1,5-benzoxazepine, 1,5-benzothiazepine and 1,5-benzodiazepine[10]. Given this, it was deemed useful to add moieties and 1,2,4-triazolo fused pyrazole and isoxazole to the previously constructed bioactive heterocyclic scaffolds.

Due to their remarkable pharmacological properties, 1,2,4-triazolo fused isoxazole and 1,2,4-triazolo fused pyrazole are members of an important class of heterocyclic compounds that have been thoroughly investigated for potential medical applications. Synthesized pyrazole derivatives are useful as food dyes (yellow food dye), sensitizing agents, anti-analgesic, anti-tubercular, antimicrobial, anti-inflammatory, anti-hyperglycemic, anti-helminthic, anti-oxidant, herbicidal properties, anti-convulsant, anti-depressant, anti-pyretic, anti-obesity, anti-rheumatic activity, as medication for arthritis, used for erectile dysfunction and sexual dysfunction in women. In addition to their usage in horticulture and agriculture, these applications have spurred interest in pyrazole chemistry[11,12].

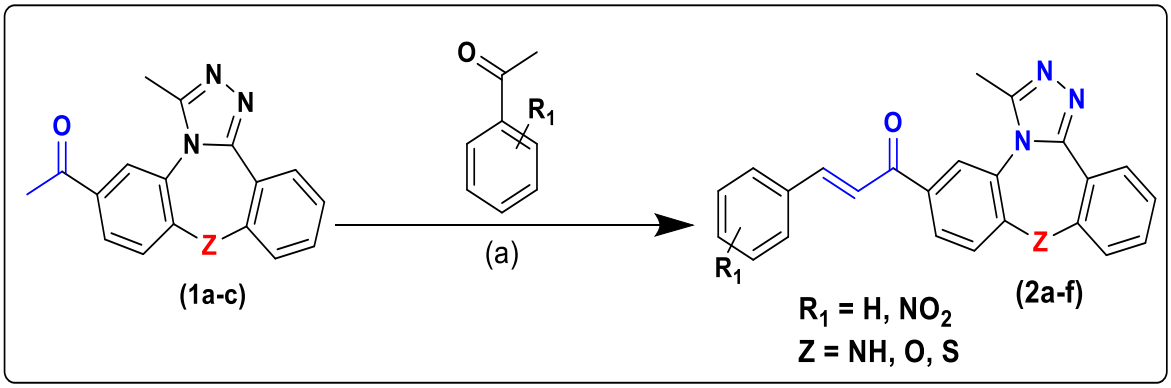
Comprising a multitude of pharmacological properties including anti-microbial, anti-viral, anti-pyretic, anti-rheumatic drug, anti-tumor, anti-biotics, anti-depressant, anxiety disorders, Parkinson's disease, urinary tract infections, analgesic, diabetic, hypoglycemic, local anesthetic, toxic psychoactive compound and anti-helminthic, isoxazoles derivatives are among the most biologically active classes of heterocyclic compounds.



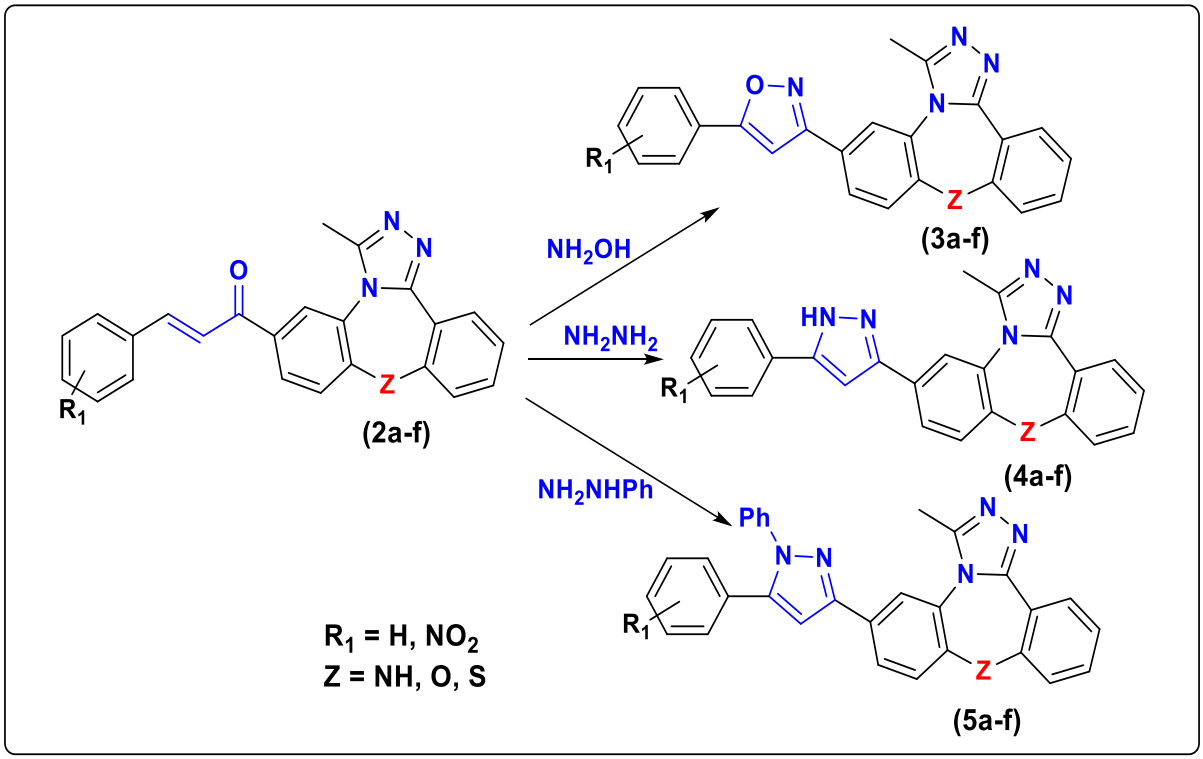
Additionally, synthesized isoxazole fused compounds have been used as adrenergic antagonists, retinoid analogs and possible fungicides. These synthesized heterocycles are notable for having weak nitrogen-oxygen bonds that in certain situations, especially in reduction (pH > 7), have the potential to be sites of ring cleavage. The ring system of isoxazoles permits the altering substitutes to yield functionally complicated derivatives; they are therefore particularly useful intermediates that can be readily cleaved as needed[1,5,6].

The creation of synthetic methods and their applications as components in the production of bioactive substances and the potential medical uses of 1,2,4-triazolo fused isoxazole and 1,2,4-triazolo fused pyrazole scaffolds have all been critically discussed in this study. Because of their many biological uses, 1,2,4-triazolo fused isoxazole and 1,2,4-triazolo fused pyrazole derivatives are among the most important groups of physiologically active compounds in synthetic and bioorganic chemistry[13,14].

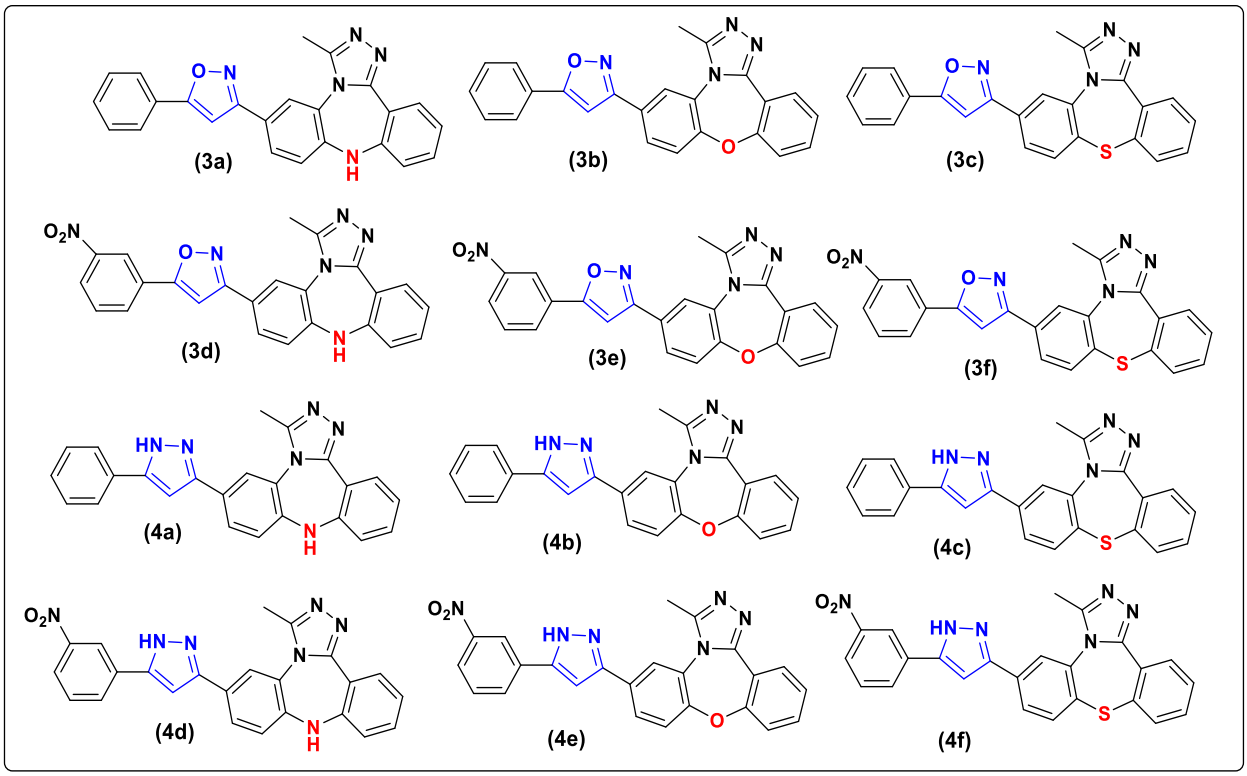
From reactive intermediate chalcones (2a-f), we synthesize [1,5]-benzothiazepine, [1,5]-benzodiazepine and [1,5]-benzoxazepine modified 1,2,4-triazolo-isoxazole and 1,2,4-triazolo-pyrazole derivatives (3a-f, 4a-f and 5a-f) (Scheme 2). Additionally, we explain how the reaction of compounds (1a-c) with different benzaldehyde derivatives yields reactive intermediate chalcones (2a-f) (Scheme 1). In the presence of sodium methoxide in methanol, chalcones (2a-f) reacted smoothly with hydroxylamine hydrochloride to yield 1,2,4-triazolo fused isoxazole derivatives (3a-f) outlined in scheme 2 and the structures of synthesized isoxazole and pyrazole derivatives are described in scheme 3.



Scheme 1: Synthesis of 1,2,4-triazolo fused chalcone Derivatives. Reaction condition and reagents: (a) 0.82 g sodium acetate, 1.06 mL benzaldehyde, reflux 6-7 h in reduced pressure.



Scheme 2: Synthesis of 1,2,4-triazolo substituted isoxazole and pyrazole derivatives.



Scheme 3: Molecular structure of synthesized pyrazole and isoxazole scaffolds.

Material and Methods

Materials: All the chemicals and required solvents were acquired from Alfa-Aesar and Sigma-Aldrich. All the chemicals were distilled, dried and were of analytical quality. We used distilled hexane, benzene, DMF and ether for TLC, IR, NMR, LC-MS and CHNS. Bruker FT-IR, AVANCE II 400 NMR, Q-TOF Micromass (LC-MS) and CHNS analyzer were used to record the spectra. On thin-layer chromatography (TLC) with pre-coated aluminum sheets and GF254 silica gel in different solvent systems, all chemicals are uniform and single spotted. All of the compounds' analytical results were discovered to be in agreement with the structures given to these molecules[18].

Synthesis of 1,2,4-triazolo-1,5-benzothiazepine fused chalcone derivative (2c): 1,2,4-triazolo derivative 1c (0.307g, 0.001mole), fused sodium acetate (0.82g, 0.01mole) and benzaldehyde (1.06 mL, 0.01mole) were refluxed in glacial acetic acid for a duration of 6-7 hours. After cooling, the reaction mixture was added to crushed ice water. Following filtration, a water wash and recrystallization from ethanol, the resultant solid gave pure compound 2c: Yield (60%) and m.p. 247–250^{0}C. In a similar manner, compounds 2(a,b and d-f) were created by reacting 1(a-f) with either 3-nitrobenzaldehyde or benzaldehyde[3].

Synthesis of 1,2,4-triazolo fused isoxazole derivative 3c from chalcone derivative 3c: Chalcone (2c) (0.395g, 0.001 mole), sodium acetate (0.82g, 0.01) and hydroxylamine hydrochloride (0.69g, 0.01 mole) were combined in 25 mL of absolute ethanol and the reaction mixture was refluxed for four to five hours. Reduced pressure was used to evaporate the methanol and the residue was then added to ice-cold water. After separation, the solid 3c was filtered and dried. Compound 3(a, b and d–f) were prepared using the same process with hydrazine hydrate[7,8].

Synthesis of 1,2,4-triazolo fused pyrazole derivatives 4c and 5c from chalcone derivative 2c (4c): 50ml of ethanol was used to reflux phenyl hydrazine hydrate (0.5mL, 0.01mole) and chalcone derivative 2c (0.395g, 0.001mole) for four to five hours. Using a rotator evaporator, the solvent was extracted under low pressure and the residue was then placed in crushed ice. After separation, the solid was filtered and dried. The pure steel grey powder 4c was obtained through recrystallization from ethanol. The production of compounds 4(a, b and d-f) and 5 (a-f) using hydrazine hydrate or phenyl hydrazine hydrate was done in the same way[4].

Characterization: The structures of all the compounds were identified by means of spectrum data obtained from elemental analysis, 1H NMR, IR and MS. It was verified that the physical data for each chemical was consistently correct for the assigned structures[9].

1,2,4-triazolo-1,5-benzothiazepine fused chalcone derivative (2c): (Yield: 75%, m.p. 255-257 ^{o}C); IR (KBr): 1085 C-S-C str., 1645 C=N str., 1630 C=O str., 1541 C=C str., 1334 C-N str.; ^{1}H-NMR (DMSO-d_6): 8.13 (d, 1 Ar-H), 8.07 (s, 1 Ar-H), 7.69 (d, 1 CH), 7.62-7.75 (m, 4 Ar-H), 7.55-7.58 (m,4 Ar-H); MS: m/z 395.01 (M^+), 394.04, 260, 130; Anal. Caled for $C_{24}H_{17}N_3OS$: C, 72.79; H, 4.43; N, 10.33; S, 08.31.

1,2,4-triazolo-1,5-benzothiazepine fused isoxazole derivative (3c): (Yield: 69%, m.p. 260°C-265°C); IR (KBr): 1081 (C-S-C), 3250 (N-H str.), 1541 (C=C str.), 1541 (C=N str.), 907 (C-O str.), 835 (O-N str.) 752 (O-N out of plane bending).; ^{1}H-NMR (DMSO-d_6): 7.52 (d, 1 ArH), 9.49 (d,1 ArH), 6.57-6.23 (m, 5 ArH), 2.50 (s, 3 CH_3); MS: m/z 407.88 (M^+), 306, 238; Anal. Caled for $C_{24}H_{16}N_4OS$: C, 70.47; H, 3.91; N, 13.72, S, 07.84.

1,2,4-triazolo-1,5-benzothiazepine fused pyrazole derivative (4c): (Yield: 78%, m.p. 287°C-290°C); IR (KBr): 3289 (N-H str.), 1540 (C=C str.), 1600 (C=N str.), 1252 (C N str.), 1142 (N-N str.); ^{1}H-NMR (DMSO-d_6): 11.37 (s, 1 NH), 7.70 (d, 1 ArH), 7.47 (1H, d, ArH), 7.22-7.55 (m, 4 ArH), 7.70-7.84 (m, 5 ArH); MS: m/z 407 (M^+), 385, 320, 250; Anal. Caled for $C_{24}H_{17}N_5S$: C, 70.74; H, 4.21; N, 17.19; S, 07.89.

1,2,4-triazolo-1,5-benzothiazepine fused pyrazole derivative (5c): (Yield: 75%, m.p. 285°C-288 °C); IR (KBr): 1405 (⎓ str.), 3288 (N-H str.), 1638 (C=N str.), 1081 (C-S-C), 1252 (C-N str.), 1155 (N-N str.); ^{1}H-NMR (DMSO-d_6): 7.30-7.42 (m, 4 ArH), 7.05-7.48 (m, 5 ArH), 8.20 (s, 5 ArH), 7.62 (d, 1 ArH), 8.25 (d, 1 ArH), 2.50 (s, 3 CH_3); MS: m/z 483 (M^+), 350, 300, 180; Anal. Caled for $C_{30}H_{21}N_5S$: C, 74.51; H, 4.38; N, 14.48; S, 06.63.

Pharmacological evaluation: Because these nuclei are the most active pharmacophores in drug design and synthesis, scientists have focused on substituted heterocyclic derivatives containing 1,2,4-triazolo-1,5-benzodiazepines, 1,2,4-triazolo-1,5-benzoxazepines and 1,2,4-triazolo-1,5-benzothiazepines fused isoxazole and pyrazole derivatives. These synthetic compounds' *in vitro* anti-microbial screening was evaluated by comparing them to two common medications, streptomycin and fluconazole. The antibacterial and antifungal properties of the recently synthesized 1,2,4-triazolo fused isoxazole and pyrazole scaffolds were evaluated by screening them against a variety of randomly selected bacterial and fungal strains including *Fusarium oxysporum, Candida albicans, Staphylococcus aureus and Escherichia coli.*

Using the disc diffusion method, the effects of the synthesized chemicals on these strains were investigated. In order to create the stock solution for the compounds to be tested, 10 mg of the compounds were first dissolved in 10 mL of DMSO. Subsequently, several dilutions at 8, 4 and 2 mg/mL were made. Table 1 is a tabular presentation of the anti-microbial study outcomes[15-17]. Comparing the compounds 3c, 4c and 5c to the conventional medication straptomycin, the anti-bacterial screening revealed that the compounds had the highest level of activity against *Escherichia coli*, *Staphylococcus aureus* and *Enterobacter cloacae*.

The findings showed that an increase in compound concentration boosted the compounds' antibacterial and antifungal effectiveness. When the chemical concentration was lowered, a consistent decline in activity was seen. According to the antibacterial test results, all of the compounds had more antibacterial activity against *S. aureus and E. coli than against E. cloacae*. The antifungal screening revealed that compounds 3c, 4c and 5c exhibited the highest levels of activity against *Fusarium oxysporum and Candida albicans*[19-21].

Table 1
Anti-bacterial and anti-fungal activity of 1,2,4-triazolo-isoxazole & 1,2,4-triazolo-pyrazole derivatives by disc diffusion method (Concentration in mg/mL).

	Concentration in (mg/mL)	***E. coli* % activity**	***E. cloacae* % activity**	***S. aureus* % activity**	***Candida albicans* % activity**	***Fusariumoxysporum* % activity**
2c	8	60.01±1.67	43.49±0.83	70.00±1.11	59.19±0.99	65.46±1.54
	4	45.31±1.11	37.04±1.09	65.95±1.18	45.35±0.11	54.37±1.62
	2	33.37±1.12	31.7±0.59	59.99±1.06	30.00±1.00	30.12±1.87
3c	8	75.60±0.30	50.56±1.50	78.15±0.20	79.21±0.48	79.66±1.40
	4	65.63±0.03	53.25±1.08	68.66±0.54	65.82±0.02	65.52±0.55
	2	50.06±0.56	54.41±1.47	65.09±0.43	40.05±0.55	40.49±0.87
4c	8	85.30±0.99	88.21±0.21	92.10±1.11	63.56±0.86	85.1±0.11
	4	80.12±0.64	70.08±0.07	87.11±0.85	62.09±0.97	56.62±0.02
	2	51.05±0.03	62.14±0.09	79.05±0.5	60.20±0.50	29.99±0.07
Streptomycin	8	28 (100)	30 (100)	25 (100)	NA	NA
	4	22 (100)	23 (100)	21 (100)		
	2	16 (100)	17 (100)	15 (100)		
Fluconazole	8	NA	NA	NA	26 (100)	25 (100)
	4				18 (100)	20 (100)
	2				5 (100)	8 (100)

The percentage activity of synthesized compounds was compared to standard Streptomycin (Antibactirial) and Fluconazole (Antifungal) at two distinct concentrations (means±SD). N.A.: Not applicable; SD. Standard Deviation[22,23].

Results and Discussion

Using hydroxylamine hydrochloride, hydrazine hydrate and phenyl hydrazine respectively, chalcones were cyclocondensed to create 1,2,4-triazolo fused isoxazole and 1,2,4-triazolo fused pyrazole derivatives. Therefore, in the presence of a base, 2(a-f) were allowed to react with hydroxylamine hydrochloride, yielding the corresponding isoxazole derivatives 3(a-f) in good proportion. Likewise, 2(a-f) produced the respective pyrazole derivatives (4a-f and 5a-f) via a smooth reaction with hydrazine hydrate and phenyl hydrazine.

2c: The IR spectra of chalcone intermediate 2c on KBr pellets showed peaks at 1085 (C-S-C str), 1630 (C=O str) and 1541 (C=C str). The peak at 1541 (C=C str.) cm^{-1} indicated the production of α and β unsaturated molecules. In the region of δ 8.07-7.58 ppm, the 1H NMR spectra of chalcone derivative 2c in $CDCl_3$ showed evidence for the presence of 17 protons, 12 of which were bonded to the carbon atoms of three aromatic rings. For the CH=CH bond, the doublet at δ 7.69 confirmed the creation of the chalcone group at and 7.71.

3c: Peaks in compound 3c's infrared spectra were seen at 3250 (N-H str.), 1541 (C=N str.), 907 (C-O str.) and 835 (O-N str.). The pyrazole ring's at a new peak at 752 (O-N out of plane bending) cm^{-1} was a clear indication that isoxazole derivative 3c had formed. Compound 3c's 1H NMR spectra in $CDCl_3$ showed evidence for the presence of 16 protons, 12 of which were attached to the carbon atoms of three aromatic rings and were found in the 7.84-6.70 ppm range. The proton of the triazolo ring and isoxazole formation was confirmed by the 3H of CH_3 singlet detected at δ 2.50 ppm. The creation of an isoxazole derivative from chalcones 2c using hydroxyl amine hydrochloride was further supported by the compound 3c's MS spectra, which showed a prominent peak at 407.88 $(M+)^{22}$.

4c: Compound 4c's infrared spectra on KBr pellets showed maxima at 3289 (N-H str.), 1600 (C=N str.) and 1252 (C-N str.). A new peak that appeared at 1142 (N-N str.) cm^{-1} was a definite sign that compound 4c had formed. The 1H NMR spectra of compound 1,2,4-triazolo fused pyrazole 4c showed signals in the range of δ 7.84-7.22 ppm that corresponded to 12 protons bound to the carbon atoms of three aromatic rings. At δ 11.37 ppm, one singlet for the 1H of the pyrazole ring was detected. Major peaks were found at 407 (M+), 385, 320 and 200 in the mass spectrometric measurements, which are given in m/z.

Conclusion

Novel 1,2,4-triazolo fused isoxazole and pyrazole derivatives were prepared in the current study and were then characterized using spectral data from the 1H NMR, IR and MS. Compounds 2c, 3c, 4c and 5c showed promising antibacterial and antifungal activities when compared to the industry standards (Streptomycin for antibacterial activities, Fluconazole for antifungal activities). The existence of antimicrobial properties in synthesized compounds was said to be responsible for the activity.

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